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Publication Details

Lian, J., Huang, X., Pai, N. & Deng, C. 2013, 'Effects of olanzapine and betahistine co-treatment on serotonin transporter, 5-HT_{2A} and dopamine D₂ receptor binding density', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 47, pp. 62-68.

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Abstract

Olanzapine is widely used in treating multiple domains of schizophrenia symptoms but induces serious metabolic side-effects. Recent evidence has showed that co-treatment of betahistidine (a histaminergic H₁ receptor agonist and H₃ receptor antagonist) is effective for preventing olanzapine-induced weight gain/obesity, however it is not clear whether this co-treatment affects on the primary therapeutic receptor binding sites of olanzapine such as serotonergic 5-HT_{2A} receptors (5-HT_{2AR}) and dopaminergic D₂ receptors (D_{2R}). Therefore, this study investigated the effects of this co-treatment on 5-HT_{2AR}, 5-HT transporter (5-HTT) and D_{2R} bindings in various brain regions involved in antipsychotic efficacy. Female Sprague Dawley rats were administered orally (t.i.d.) with either olanzapine (1 mg/kg), betahistidine (2.7 mg/kg), olanzapine plus betahistidine (O + B), or vehicle (control) for 2 weeks. Quantitative autoradiography was used to detect the density of [³H]ketanserin, [³H]paroxetine and [³H]raclopride binding site to 5-HT_{2AR}, 5-HTT and D_{2R}. Compared to the controls, olanzapine significantly decreased [³H]ketanserin bindings to 5-HT_{2AR} in the prefrontal cortex, cingulate cortex, and nucleus accumbens. Similar changes in 5-HT_{2AR} bindings in these nuclei were also observed in the O + B co-treatment group. Olanzapine also significantly decreased [³H]paroxetine binding to 5-HTT in the ventral tegmental area and substantia nigra, however, both olanzapine only and O + B co-treatment did not affect [³H]raclopride binding to D_{2R}. The results confirmed the important role of 5-HT_{2AR} in the efficacy of olanzapine, which is not influenced by the O + B co-treatment. Therefore, betahistidine co-treatment would be an effective combination therapy to reduce olanzapine-induced weight gain side-effects without affecting olanzapine's actions on 5-HT_{2AR} transmissions.

Keywords

binding, receptor, d₂, dopamine, ht_{2a}, 5, density, effects, transporter, olanzapine, betahistidine, co, treatment, serotonin

Disciplines

Medicine and Health Sciences

Publication Details

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Effects of olanzapine and betahistine co-treatment on serotonin transporter, 5-HT_{2A} and dopamine D₂ receptor binding density

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Source of Funding:

This study was supported by a grant from the Australian National Health and Medical Research Council (APP1027493).

Abstract

Olanzapine is widely used in treating multiple domains of schizophrenia symptoms but induces serious metabolic side-effects. Recent evidence has showed that co-treatment of betahistine (a histaminergic H₁ receptor agonist and H₃ receptor antagonist) is effective for preventing olanzapine-induced weight gain/obesity, however it is not clear whether this co-treatment affects on the primary therapeutic receptor binding sites of olanzapine such as serotonergic 5-HT_{2A} receptors (5-HT_{2A}R) and dopaminergic D₂ receptors (D₂R). Therefore, this study investigated the effects of this co-treatment on 5-HT_{2A}R, 5-HT transporter (5-HTT) and D₂R bindings in various brain regions involved in antipsychotic efficacy. Female Sprague Dawley rats were administered orally (t.i.d.) with either olanzapine (1mg/kg), betahistine (2.7mg/kg), olanzapine plus betahistine (O+B), or vehicle (control) for 2-weeks. Quantitative autoradiography was used to detect the density of [³H]ketanserin, [³H]paroxetine and [³H]raclopride binding site to 5-HT_{2A}R, 5-HTT and D₂R. Compared to the controls, olanzapine significantly decreased [³H]ketanserin bindings to 5-HT_{2A}R in the prefrontal cortex, cingulate cortex, and nucleus accumbens. Similar changes in 5-HT_{2A}R bindings in these nuclei were also observed in the O+B co-treatment group. Olanzapine also significantly decreased [³H]paroxetine binding to 5-HTT in the ventral tegmental area and substantia nigra, however, both olanzapine only and O+B co-treatment did not affect [³H]raclopride binding to D₂R. The results confirmed the important role of 5-HT_{2A}R in the efficacy of olanzapine, which is not influenced by the O+B co-treatment. Therefore, betahistine co-treatment would be an effective combination therapy to reduce olanzapine-induced weight gain side-effects without affecting olanzapine's actions on 5-HT_{2A}R transmissions.

Keywords: Olanzapine, betahistine, receptor binding, 5-HT_{2A} receptor, 5-HT transporter, dopamine D₂ receptor

List of Abbreviations

5-HT	Serotonin
5-HT _{2A} R	Serotonin _{2A} receptor
5-HTT	Serotonin transporter
α_{1-2} R	α_{1-2} receptor
ANOVA	Analysis of variance
Cg	Cingulate cortex
D ₂ R	Dopamine D ₂ receptor
CPu	Caudate putamen
EPS	Extrapyramidal symptoms
FGAs	First generation antipsychotic drugs
H ₁ R	Histamine H ₁ receptor
H ₃ R	Histamine H ₃ receptor
M ₁ R	M ₁ Receptor
NAcC	Nucleus accumbens, core
NAcS	Nucleus accumbens, shell
PFC	Prefrontal cortex
RT	Room temperature
SGAs	Second generation antipsychotic drugs
SN	Substantia nigra
VTA	Ventral tegmental area

1. Introduction

Second generation antipsychotic drugs (SGAs) such as olanzapine have been used not only to ameliorate schizophrenia symptoms but also used widely for other mental disorders such as bipolar disorder, depression, and Tourette's syndrome (Lambert, 2011). However, these drugs could induce serious metabolic side-effects including body weight gain and obesity (Allison et al., 1999, Stahl et al., 2009, Correll et al., 2011). In contrast to first generation antipsychotic drugs (FGAs) (e.g. chlorpromazine and haloperidol) as a potent dopamine D₂ receptor (D₂R) antagonist, SGAs (such as olanzapine) have high affinities to not only D₂R but also to a wide range of non-dopaminergic G-protein-coupled receptors, including histaminergic H₁ (H₁R), serotonergic 5-HT_{2A} (5-HT_{2A}R) and 5-HT_{2C} (5-HT_{2C}R), muscarinic M₁ (M₁R) and adrenergic α_{1-2} (α_{1-2} R) receptors (Richelson and Souder, 2000, Nasrallah, 2008). The binding affinities of olanzapine to these neurotransmitter receptors are ranked in the following order: H₁R>5-HT₂R>D₂R> α R and M₁R (Uchida et al., 2007). Since a number of studies have shown that H₁R plays a key role in olanzapine induced weight gain/obesity (Kim et al., 2007, Han et al., 2008a, Deng et al., 2010, He et al., 2013), H₁R could be a therapeutic target for controlling olanzapine-induced weight gain side-effects. Recently we found in a rat model that olanzapine-induced weight gain can be partially reduced (-45%) by co-treatment with betahistine (an H₁R agonist and H₃R antagonist) (Deng et al., 2012). This finding was confirmed by a recent clinical report that schizophrenia patients with a combination treatment of olanzapine, betahistine and reboxetine (a selective norepinephrine reuptake inhibitor) had significantly less weight gain than those on olanzapine only (Poyurovsky et al., 2013). In addition, a small clinical trial with 3 first episode schizophrenic patients also found that betahistine was able to prevent weight gain related to olanzapine treatment (Poyurovsky et al., 2005). Therefore, current evidence confirms the potential of betahistine in preventing the weight gain/obesity side-effect caused by olanzapine and

possibly other SGAs. One important issue that should be addressed, however, is whether co-treatment of olanzapine and betahistine affects the antipsychotic efficacy of olanzapine.

D₂R is proven to be a key receptor for the pathophysiology of schizophrenia and the therapeutic effects of antipsychotic drugs (Kapur et al., 1999, Kapur and Mamo, 2003, Stahl, 2003, Volk, 2010, Seeman, 2011), and also plays an important role in the reward function (Volkow et al., 2011). Dopaminergic neurons have major projections derived from the mesencephalon including (1) the nigrostriatal pathway, in which the substantia nigra (SN) projects to the caudate putamen (CPu); (2) the mesolimbic pathway, in which the ventral tegmental area (VTA) projects to the nucleus accumbens (NAc); and (3) the mesocortical pathway, in which the VTA projects to the cortex including the PFC and Cg (Kapur and Mamo, 2003, Volk, 2010). Accumulated evidence suggests that D₂R is the common target for all of current antipsychotics (Kapur et al., 1999, Kapur and Mamo, 2003, Seeman, 2011). On the other hand, it has been proposed that a relatively higher 5-HT_{2A}R affinity compared to the D₂R is the mechanism underlying the therapeutic effects of SGAs (Meltzer et al., 2003, Horacek et al., 2006, Kuroki et al., 2008, Meltzer and Massey, 2011). Serotonin neurons are located in the raphe nuclei which broadly project to various brain regions and exert their actions via various types of 5-HT receptors (Millan et al., 2008). Among them, 5-HT_{2A}R plays a critical role in the action of SGAs such as olanzapine (Meltzer and Massey, 2011). Moreover, the clinical data showed that there was decreased 5-HT_{2A}R binding in the prefrontal cortex (PFC), and cingulate cortex (Cg), superior temporal gyrus and striatum in post-mortem brains of schizophrenia patients treated by SGAs (Matsumoto et al., 1996, Scarr et al., 2004, Steward et al., 2004, Kang et al., 2009).

Furthermore, the blockade of 5-HT_{2A}R by olanzapine could contribute to the 5-HTergic modulation on the nigrostriatal, mesolimbic or mesocortical dopaminergic pathways which affects dopaminergic output in the NAc, CPu, PFC and Cg (Van Oekelen et al., 2003, Horacek et al., 2006) and D₂R mediated neurotransmission (Meltzer and Massey, 2011). Therefore, due to the critical roles of 5-HT_{2A}R and D₂R in the therapeutic effect of SGAs, this study examined the effects of olanzapine and/or betahistine treatment on 5-HT_{2A}R, and D₂R bindings in the key brain regions for therapeutic effect of SGAs. In addition, the binding density of serotonin transporter (5-HTT) has also been examined.

2. Methods

2.1. Animal treatment and administration

Female Sprague Dawley rats (201-205g) were obtained from the Animal Resources Centre (Perth, WA, Australia). After one week of environmental familiarisation, they were housed in individual cages and allowed *ad-libitum* access to water and standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate and 16% protein) under environmentally controlled conditions (22°C, light cycle from 07:00 to 19:00 and dark cycle from 19:00 to 07:00) throughout the whole study (Han et al., 2008a, Deng et al., 2012, Weston-Green et al., 2012). Rats were randomly administered different drugs 3 times per day orally (07:00, 15:00, 23:00 h) (n=12) for two weeks: (1) olanzapine only (1mg/kg, Eli Lilly, USA), (2) betahistine only (2.67 mg/kg, Manus Aktteva, India), (3) combined olanzapine and betahistine (O+B), or (4) control (vehicle) (Deng et al., 2012). Drugs were prepared in advance by mixing with cookie dough pellets (0.3g; including 30.9% cornstarch, 30.9% sucrose, 6.3% gelatine, 15.5% casein, 6.4% fibre, 8.4% minerals and 1.6% vitamins) and droplets of water (Han et al., 2008a, Deng et al., 2012, Weston-Green et al., 2012). The equivalent pellet without drug was

used as controls. Prior to the drug treatment, rats were trained with cookie-dough pellets without any drug for one week. The half life of olanzapine in rats is 2.5 and 5.1 h in the plasma and brain respectively, however the drug concentration maintains a high level after 8 h in the rat brain, compared with the half-life of 24.2 and 72 h in human plasma and brain (Aravagiri et al., 1999, Tauscher et al., 2002). Therefore, 8 hour intervals were chosen in the present study in order to maintain the high concentration of olanzapine and to mimic clinical administration (Deng et al., 2012). All experimental procedures have been approved by the Animal Ethics Committee, University of Wollongong, Australia (AE11/10); and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Body weight and energy intake data have been reported previously that, in brief, olanzapine only treatment induced significant body weight gain and increased food intake, while co-treatment of olanzapine with betahistine significantly prevented (-45%) weight gain and reduced feeding efficiency compared to sole olanzapine treatment (Deng et al., 2012). Six brains from each group were used for binding experiments.

2.2. Histological procedures

All rats were sacrificed using carbon dioxide asphyxiation, and then brains were removed and frozen in liquid nitrogen immediately followed by storage in a -80 °C freezer until sectioning. Brains were cut at -18 °C into 14 µm coronal sections using a cryostat (Leica CM1850, Leica Microsystems, Germany). The corresponding brain regions were obtained based on a standard rat brain atlas (Paxinos and Watson, 2007) (Figure 1). Brain sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and stored at -20 °C. A set of sections from each animal was stained with 0.5% cresyl violet solution (Nissl staining) and used for confirmation of anatomical structures.

2.3. Receptor binding autoradiography and quantification

The procedures for the 5-HT_{2A}R, 5-HTT, and D₂R binding autoradiography have been reported previously (du Bois et al., 2006, Huang et al., 2006b, du Bois et al., 2008, Kang et al., 2009, Kesby et al., 2012).

2.3.1. 5-HT_{2A}R binding procedures

In brief, brain sections containing the PFC, Cg, NAc, CPu, VTA, and SN were thawed at room temperature (RT), and after 15 min of pre-incubation in 170 mM Tris buffer (pH 7.4), they were incubated with 2nM [³H]ketanserin (Specific activity: 67 Ci/mMol; PerkinElmer, USA) in 170 mM Tris buffer for 2 h at RT to determine total binding of 5-HT_{2A}R. Non-specific binding was determined by incubating the next sequential sections with 2nM [³H]ketanserin incubation buffer, with the addition of 2μM spiperone (Sigma Pharmaceuticals, Australia). Slides were washed twice for 10 minutes in ice-cold buffer, dipped in ice-cold distilled water, and then dried under a stream of cool air to remove excess buffer salts (du Bois et al., 2006, Kang et al., 2009).

2.3.2. 5-HTT binding procedures

Binding of [³H]paroxetine (specific activity: 20.8 Ci/mmol, PerkinElmer, USA) to 5-HTT was performed based on procedures previously described (du Bois et al., 2006). In brief, sections were pre-incubated in 50 mM Tris buffer including 120 mM NaCl and 5 mM KCl (pH 7.4) for 15 min at RT. Sections were then incubated for 2 h at RT in the same buffer containing 0.6 nM [³H]paroxetine for the total binding. Non-specific binding was determined with the addition of 10 μM fluoxetine. After incubation, the sections were washed in ice-cold buffer (2×10 min), dipped in distilled water and air dried (du Bois et al., 2006).

2.3.3. D₂R binding procedures

In terms of the D₂R binding, the 50 mM Tris buffer added with 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1mM MgCl₂ and 0.001% ascorbic acid (pH 7.4) was applied for a pre-incubation period of 30 min. Furthermore, 5 nM [³H]raclopride (specific activity: 60.1 Ci/mmol; PerkinElmer, USA) was used for total D₂ receptor binding during 1 h of incubation, while non-specific binding was determined by 10 µM butaclamol and 5 nM [³H]raclopride using the same buffer. After incubation, the slides were washed with the cold buffer (2×5 min), dipped in distilled water and air dried (Huang et al., 2006b, du Bois et al., 2008, Kesby et al., 2012).

2.3.4. Quantification

The receptor binding image was captured using a Beta-imager™ 2000 (BioSpace, Paris, France) with a high-resolution setting and scanned for 3.5 hours. One slide with a known amount of radioactivity was used as a standard (Deng et al., 2007, Kang et al., 2009). The level of bound radioactivity in the brain sections was determined by directly counting the number of β-particles emitted from the tissue, which was analysed using a Beta Vision Plus programme (Version 4, BioSpace, Paris, France). The radioligand binding signal was converted from counts per minute per square millimetre (cpm/mm²) to nanocuries per milligramme (nCi/mg) of tissue equivalent (nCi/mg TE) (Deng et al., 2007, Kang et al., 2009). Specific binding was calculated by subtracting non-specific binding from total binding. Specific brain regions in this project were identified by reference to the Nissl-stained sections and a standard rat brain atlas (Paxinos and Watson, 2007).

2.4. Statistical analysis

Statistical analysis was performed using SPSS (IBM version 19.0, SPSS Inc., USA). Two-way ANOVAs (OLANZAPINE $\times$ BETAHISTINE) were used to analyse receptor binding density in relevant brain regions of the rat. The post-hoc Dunnett-T tests were followed for comparison between groups. All data are expressed as mean $\pm$ SEM, and statistical significance will be accepted when $p < 0.05$.

3. Results

3.1. 5-HT_{2A}R binding

Examples of [³H]ketanserin binding to 5-HT_{2A}R are presented in Figure 1 (A'-C'). Two-way ANOVAs revealed the significant main effect of OLANZAPINE factor in the PFC ($F_{1,20}=78.060$, $p < 0.001$), Cg ($F_{1,20}=66.025$, $p < 0.001$), NAcC ($F_{1,20}=14.574$, $p = 0.001$), and NAcS ($F_{1,20}=11.203$, $p = 0.003$), but no effect of the BETAHISTINE factor (all $p > 0.05$) and also no significant interactions between the two factors in these brain regions (all $p > 0.05$). Post-hoc analysis demonstrated that compared to the control (Figures 2 and 3), olanzapine significantly decreased the levels of 5-HT_{2A}R binding density in the PFC ($p < 0.001$), Cg ($p < 0.001$), as well as NAcC ($p < 0.05$) and NAcS ($p < 0.05$). Similarly, the O+B co-treatment also significantly decreased 5-HT_{2A}R binding density in the PFC ($p < 0.001$; Figures 2 and 3), Cg ($p < 0.001$), NAcC ($p < 0.01$), and NAcS ($p < 0.05$). However, olanzapine and betahistine had no effect on 5-HT_{2A}R binding density in the CPu (Figure 3C) and VTA (Figure 3F). In the SN, although olanzapine had a lower 5-HT_{2A}R binding than the control group, it was not significant ($p > 0.05$; Figure 3F).

3.2. 5-HTT binding

Examples of [³H]paroxetine binding to 5-HTT are presented in Figure 1 (A''-C''). Two-way ANOVAs revealed a significant effect of the OLANZAPINE factor on 5-HTT binding in the VTA ($F_{1,20}=9.728$, $p=0.005$) and SN ($F_{1,20}=12.445$, $p=0.002$), but no significant effect of the BETAHISTINE factor (all $p>0.05$) and also no significant interactions between the two factors in these brain regions (all $p>0.05$). Post-hoc analysis showed that, olanzapine treatment significantly decreased the 5-HTT binding density in the VTA ($p<0.05$) and SN ($p<0.01$) compared with control (Figure 4 E and F). O+B co-treatment tended to decrease 5-HTT binding density in the SN ($p=0.061$; Figure 4F). In the VTA (Figure 4E), O+B group had a lower 5-HTT binding density than the control, but not significant ($p>0.05$). However, there were no significant differences between the control group and all drug treatment groups in other brain regions (Figure 4 A-D).

3.3. D₂R binding

Examples of [³H]raclopride binding to D₂R were presented in Figure 1 (A'''-C'''). Two-way ANOVAs found no significant effects of the OLANZAPINE and BETAHISTINE factors in all brain regions examined (all $p>0.05$). Moreover, no significant interaction between the two factors was detected in these brain regions (Table 1).

4. Discussion

This study investigated the effects of olanzapine and/or betahistine treatment on 5-HT_{2A}R, 5-HTT, and D₂R binding density in the rat brain. Both olanzapine only and O+B co-treatment down-regulated 5-HT_{2A}R binding density in various brain regions, including the PFC, Cg, NAcC and NAcS. In terms of the 5-HTT binding, compared with control, olanzapine

treatment significantly decreased the binding density in the VTA and SN. However, no significant effects of olanzapine and/or betahistine treatment were detected on D₂R binding.

It has been repeatedly reported that treatment with SGAs such as olanzapine and clozapine causes down-regulation of 5-HT_{2A}R in receptor binding, protein level and mRNA expression, which contributes to the therapeutic efficacy of these SGAs (Kusumi et al., 2000, Tarazi et al., 2002, Huang et al., 2006a, Kuroki et al., 2008, Yadav et al., 2011). Consistent with these reports, this study found that olanzapine only treatment decreased 5-HT_{2A}R bindings in brain regions involved in antipsychotic therapeutics including the PFC, Cg, NAcC and NAcS (Tarazi et al., 2002, Cohen et al., 2003, Stahl, 2003, Yadav et al., 2011). It is important that O+B co-treatment caused the same down-regulation in 5-HT_{2A}R binding density as olanzapine only treatment, while betahistine only treatment had no significant effects on 5-HT_{2A}R bindings in these brain regions. These results suggest that O+B co-treatment should have similar down-regulation effects on 5-HT_{2A}R as olanzapine only treatment.

Although several studies have identified an association between polymorphism of the 5-HTT gene and therapeutic effects of olanzapine in schizophrenia patients (Mata-Pastor et al., 2002, Bozina et al., 2007, Vazquez-Bourgon et al., 2010), it is not clear how 5-HTT contributes to the response to olanzapine treatment in schizophrenia. To our knowledge, this is the first study to examine the effects of olanzapine on 5-HTT bindings, in which we found olanzapine decreased 5-HTT bindings in the SN and VTA. O+B group had similar tendency in decreasing 5-HTT bindings in this region. This result suggests that olanzapine may act through modulating 5-HT uptake in the SN and VTA, two key regions for antipsychotic therapeutics (Stahl, 2003, Strange, 2008).

In this study, olanzapine treatment did not cause any significant changes in D₂R bindings in all brain regions. This result confirmed previous reports that both short-term and chronic olanzapine treatment did not affect D₂R bindings in the PFC, CPu and NAc (Kusumi et al., 2000, Han et al., 2009a). Similarly it has been reported that SGAs with lower affinity to D₂R such as clozapine and quetiapine did not affect D₂R bindings, while antipsychotics with high D₂R affinity such as haloperidol, chlorpromazine and risperidone have been reported to increase D₂R bindings (Tarazi et al., 1997, Kusumi et al., 2000, Tarazi et al., 2001). These results are consistent with a PET study showing that olanzapine had a lower D₂R blockade compared to haloperidol in schizophrenia patients (Xiberas et al., 2001). Although one study observed that olanzapine increased D₂R density in the PFC, NAc and CPu of rats, much higher doses of olanzapine (5.0 mg/kg/day compared to 1 mg/kg, t.i.d. in this study) and osmotic minipumps were used in that study (Tarazi et al., 2001). However, a recent study has provided clear evidence that osmotic minipumps are not a suitable method for olanzapine delivery due to a rapid degradation of olanzapine in water solution (Remington et al., 2011). It is worth noting that olanzapine could modulate dopamine release in the PFC, NAc and CPu through 5-HT_{2A}R (Li et al., 1998, Marcus et al., 2000, Kuroki et al., 2008). In the PFC, experiments showed that 5-HT_{2A}R and D₂R blockage by olanzapine increased dopamine release via 5-HT_{1A}R activation (Ichikawa et al., 2001), although in a recent study reported that olanzapine could increase dopamine release via 5-HT_{1A}R activation in 5-HT_{2A}R knockout mice (Bortolozzi et al., 2010). Therefore, although olanzapine treatment does not influence D₂R binding, there should be interactions between 5-HT_{2A}R and dopamine transmission (Kuroki et al., 2008). Furthermore, this is the first study to investigate the effects of betahistine on 5-HT_{2A}R and D₂R bindings. We found that O+B co-treatment and betahistine only treatment did not change D₂R bindings in all brain regions examined, which suggests that O+B co-treatment does not influence the action of olanzapine on D₂R

transmission. However, D₂R can exist in a state of high-affinity (D₂^{high}) or in a state of low-affinity for dopamine (D₂^{low}) (Seeman et al., 2006, Seeman, 2011). Normally, about 10-20% of D₂R population is in the D₂^{high} state (Seeman, 2011). It has been reported that long-term treatment of antipsychotics generally increases D₂R density by 10-40%, while it increases the proportion of D₂^{high} receptors by a factor of 2-4-fold (2-fold for 9-day treatment of 0.75mg/kg olanzapine) (Seeman et al., 2006). Therefore, it is important in further studies to investigate whether O+B co-treatment affects the proportion of D₂^{high} receptors.

The animal model of olanzapine-induced weight gain has been well established and validated in female rats in our and other laboratories (Goudie et al., 2002, Choi et al., 2007, Weston-Green et al., 2011, Deng et al., 2012). The sensitivity of female rats to weight gain side-effect over males is also a common observation in the clinic, where female patients have a much higher risk for weight gain side-effects associated with atypical antipsychotics (Gebhardt et al., 2009, Seeman, 2009, Weston-Green et al., 2010, Treuer et al., 2011). Furthermore, in this study, rats were closely housed in a room occupied by only female rats. Our pre-experiments have shown that, under this rearing condition, the estrus cycles of all female rats are synchronized (unpublished data). The olanzapine dosage used in this study (1 mg/kg, t.i.d.) has been shown to be pharmacologically effective in affecting central receptor systems relative to its pharmacological profile (Han et al., 2008b, Han et al., 2009a, Han et al., 2009b, Weston-Green et al., 2011), as well as behaviourally effective in affecting locomotor activity and food intake, and producing the obesity phenotype in rats (Han et al., 2008a, Weston-Green et al., 2011, Deng et al., 2012). The dosage of betahistine (2.67 mg/kg, t.i.d.) is effective in reducing food intake during acute treatment (Szelag et al., 2001) and preventing olanzapine-induced weight gain in rats (Deng et al., 2012). According to dosage translation between species based on body surface area following an FDA guideline for clinical trials

(Center for Drug Evaluation and Research FDA, 2005, Reagan-Shaw et al., 2008), 1 mg/kg olanzapine in rats is equivalent to ~10 mg in humans (60kg body weight), and 2.67 mg/kg betahistine in rats to ~26 mg in humans; both are among the recommended clinical doses.

5. Conclusions

The present study provides the first evidence that O+B co-treatment has similar effects as olanzapine only treatment on the 5-HT_{2A}R, 5-HTT and D₂R bindings in key regions of the brain associated with antipsychotic therapeutics (Kapur and Mamo, 2003, Kuroki et al., 2008). This study suggested that the co-treatment of olanzapine and betahistine could be a fair option for ameliorating the body weight gain/obesity side-effect without affecting olanzapine actions on 5-HT_{2A}R and D₂R transmissions. Consistently, a small clinical trial for 6 weeks co-treatment of olanzapine and betahistine did not observed any interference with the antipsychotic effects of olanzapine (Poyurovsky et al., 2005). This was confirmed by a 6-week trial of co-treatment of olanzapine and betahistine-reboxetine that did not interfere with olanzapine's effect on core schizophrenia symptoms (Poyurovsky et al., 2013). It is important to investigate whether co-treatment of betahistine and other SGAs will have similar effects on weight gain, 5-HT_{2A}R, 5-HTT and D₂R bindings, as well as efficacy in controlling schizophrenia symptoms.

Acknowledgements

This study was funded by the Australian National Health and Medical Research Council (APP1027493). C. Deng and X.-F. Huang are supported by the Schizophrenia Research Institute, Australia, utilising infrastructure funding from NSW Health.

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Table 1 Specific [³H]raclopride binding (nCi/mg tissue; mean values ± SEM; n=6/group) to D₂ receptors in different brain regions following olanzapine and/or betahistine treatment.

Group	Control	Olanzapine	Betahistine	O+B
PFC	1.93 ± 0.10	2.17 ± 0.07	1.98 ± 0.17	1.81 ± 0.18
Cg	3.13 ± 0.09	3.16 ± 0.10	3.24 ± 0.13	2.90 ± 0.11
CPu	3.61 ± 0.11	3.73 ± 0.12	3.71 ± 0.15	3.56 ± 0.05
NAcC	3.29 ± 0.13	3.41 ± 0.06	3.67 ± 0.17	3.26 ± 0.07
NAcS	3.03 ± 0.09	3.11 ± 0.07	3.18 ± 0.11	3.02 ± 0.14
SN	1.73 ± 0.11	1.69 ± 0.17	1.82 ± 0.09	1.48 ± 0.09
VTA	1.81 ± 0.11	1.78 ± 0.17	1.85 ± 0.15	1.59 ± 0.10

Abbreviations: Cg, cingulate cortex; CPu, caudate putamen; NAc, nucleus accumbens, core; NAs, nucleus accumbens, shell; O+B, olanzapine and betahistine co-treatment; PFC, prefrontal cortex; SN, substantia nigra; VTA, ventral tegmental area.

Figure Legends

Figure 1 Examples of 5-HT_{2A}, 5-HTT, and D₂ receptor bindings in the rat brain. A-C, the schematic diagram is adapted from a rat brain atlas (Paxinos and Watson, 2007) showing the level of Bregma 3.72 mm (A), 1.08 mm (B), and -5.04 mm (C). A'-C', examples of digital autoradiogram obtained with a Beta Imager to show [³H]ketanserin binding to 5-HT_{2A} receptors. A''-C'', examples of [³H]paroxetine binding to 5-HTT. A'''-C''', examples of [³H]raclopride binding to D₂ receptors. Abbreviations: Cg, cingulate cortex; CPu, caudate putamen; NAcC, nucleus accumbens, core; NAcS, nucleus accumbens, shell; PFC, prefrontal cortex; SN, substantia nigra; VTA, ventral tegmental area.

Figure 2 Typical autoradiographs depict the binding densities of 5-HT_{2A} receptors in the prefrontal cortex (PFC) of rats (n=6/group) treated with (A) vehicle (control), (B) olanzapine (Olan), (C) betahistine (Beta), and (D) olanzapine and betahistine co-treatment (O+B). Standard bar: min/max: 0-7 nCi/mg tissue equivalent.

Figure 3 The effects of olanzapine and/or betahistine treatment (n=6/group) on [³H]ketanserin binding to 5-HT_{2A} receptors (nCi/mg tissue) in (A) the prefrontal cortex (PFC), (B) cingulate cortex (Cg), (C) caudate putamen (CPu), (D) nucleus accumbens, core (NAcC), (E) ventral tegmental area (VTA), and (F) substantia nigra (SN). Data shown are the mean values ± SEM. * *p*<0.05, ** *p*<0.01, *** *p*<0.001 vs. control. Abbreviations: Beta, betahistine; Olan, olanzapine; O+B, olanzapine and betahistine co-treatment.

Figure 4 The effects of olanzapine and/or betahistine (n=6/group) on [³H]paroxetine binding to 5-HTT (nCi/mg tissue) in (A) the prefrontal cortex (PFC), (B) cingulate cortex (Cg), (C) caudate putamen (CPu), (D) nucleus accumbens, core (NAcC), (E) ventral tegmental area

(VTA), and (F) substantia nigra (SN). Data shown are the mean values $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. control. Abbreviations are the same as in Figure 3.

Figure 1
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Figure 2
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Figure 3

Figure 4